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RESEARCH ARTICLE **ARAŞTIRMA MAKALESİ**

Effects of perfluorooctane sulfonate compounds on the biochemical activities in mussels (*Mytilus galloprovincialis*)

Perflorooktan sülfonat (PFOS)'ın midyelerde (*Mytilus galloprovincialis*) biyokimyasal etkilerinin incelenmesi

Gizem Gülsever[1] • Hatice Parlak[2*]

[1] Ege University, Faculty of Fisheries, Marine Biology Department, Bornova/İzmir-Turkey https://orcid.org/0000-0001-7526-6207
[2] Ege University, Faculty of Fisheries, Marine Biology Department, Bornova/İzmir-Turkey https://orcid.org/0000-0002-7623-9662
Corresponding author: hatice.parlak@ege.edu.tr

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Abstract: Our environment has been affected by increasing level of discharged organic and inorganic pollutants from anthropogenic sources. Perfluorinated compounds (PFCs) are one of the important sources of pollution and they are major risks for the aquatic ecosystems. The aim of this study is to determine the effects of PFOS on GST enzyme activities in mussels (*Mytilus galloprovincialis*). For this purpose, mussels were exposed to six different PFOS concentrations and the effects were evaluated. PFOS has been caused to a statistically significant increase in GST activity in hepatopancreas in all experimental groups compared with the control group. In conclusion, it has been approved that GST which is a defense mechanism of organisms, can be a very useful tool to detect the toxic effects of pollutants.

Keywords: Perfluorinated compounds, perfluorooctane sulfonate, mussels, *Mytilus galloprovincialis*, GST enzyme activity

Öz: İnsan aktiviteleri sonucunda, çevremiz her geçen gün gittikçe artan konsantrasyonda organik ve inorganik kirleticilere maruz kalmaktadır. Bu koşullarda, sucul ekosistemler açısından büyük risk oluşturan perflorlu bileşikler önemli bir yer tutmaktadır. Çalışmanın amacı, PFOS'un midyede (*Mytilus galloprovincialis*) GST enzim aktivitesi üzerine etkilerini belirlemektir. Bu amaçla, midyeler 6 farklı PFOS konsantrasyonuna maruz bırakılmış ve etkileri değerlendirilmiştir. Sonuç olarak, midye hepatopankreasında GST enzim aktivitesinin bütün deneme gruplarında kontrole göre istatistiksel olarak anlamlı bir yükselme gösterdiği bulunmuştur ve midyelerin korunma mekanizması olarak GST'nin, kirleticilerin toksik etkilerini belirlemek için yararlı bir araç olduğu ortaya konulmuştur.

Anahtar kelimeler: Perflorlu bileşikler, perflorooktan sülfonat, midye, *Mytilus galloprovincialis*, GST enzim aktivitesi

INTRODUCTION

The aquatic environment is affected by different sources of pollution like domestic, industrial wastes and residue of human agricultural practices. Unfortunately, this ecological problem perceived as a universal problem nearly after the industrial revolution of the 1750s. The chemicals were increasingly used to enhance our daily life comfort in various fields. Researchers have paid more attention and give priority to the studies related with deleterious effects of chemicals such as persistent organic pollutants (POPs) on ecosystems, since the concentrations of organic and inorganic pollutants from anthropogenic sources in the environment have been increasing. Effects of different pollutants can be assessed by various bio tests like AMES/Salmonella mutagenicity test (Boyacıoğlu et al., 2007), embryotoxicity test with sea urchins (Karaaslan et al., 2012; Gunduz et al., 2013) or algal growth inhibition assay (Katalay et al., 2012).

POPs are the priority pollutants that pose a risk to aquatic ecosystems and human health due to biomagnification through aquatic food chain. The usage of POPs was restricted in 2004 by the Stockholm Convention. Perfluorinated compounds, particularly PFOS are widely used in the industrial applications, such as protective coatings of carpets, furniture, paper and textile as well as in the polytetrafluoroethylene products, and fire-fighting foams (Ahrens and Bundschuh 2014). Perfluoroalkyl and polyfluoroalkyl substances (PFASs) have been detected in many compartments of ecosystems due to the gross usage for almost 60 years (Houde et al., 2011; Wang et al., 2015; Yamashita et al., 2005). Some of the PFASs are listed in the national and international regulations because of bioaccumulative and toxic nature (OECD, 2002). Perfluorooctanesulfonic acid (PFOS) were added to Stockholm Convention's Annex B in 2009 and then its production and usage gradually decreased. Although, Zhao et al (2017) reported that major global manufacturers of perfluorooctanoic acid (PFOA) and its precursors were promised to voluntarily stop their production in 2015 (Zhao et.al, 2017; EPA, 2010), the releases of PFASs continue (Kwok et al., 2015; Müller et al., 2011). PFOS have strong carbon fluorine bonds and have lipophobic and hydrophilic characteristics (Kissa, 2001; Lindstrom et al., 2011). Ionic PFASs are resistant to photolysis, pyrolysis, hydrolysis and biotransformation. Thus they are highly persistent component in the environment (Kissa, 2001; Han et al., 2015).

As a result of this, PFOS has been detected in various environments such as air, sediments and water (Yeung et al, 2006; Kovarova et al., 2012; Naile et al., 2010). Paul et al. (2009) had reviewed that "total historical worldwide production of POSF was estimated to be 96 000 t (122 000 t, including unusable wastes) between 1970-2002. Estimated global release of production were 45 250 t to air and water 1970-2012 from direct (manufacture, use, and consumer products) and indirect (PFOS precursors and/or impurities) sources. The various ecological studies confirm that a large part of perfluorinated compounds were found in surface waters, especially in the oceans (Prevedouros et al., 2006). Several studies on terrestrial and aquatic species showed that these compounds lead to toxic effects in living organisms (O'Brien et al., 2009; Huang et al., 2010; Boudreau et al., 2003).

In order to find out harmful effects of pollutants, back ground information is necessary at different trophic levels. Biomarkers is considered the most promising tools for ecotoxicological applications because of their ability to identify causal mechanisms that is potentially responsible for effects at higher levels of organization (Peakall and Walker, 1994; Adams, 2002). Generally, GST enzyme activities are included in this group of biomarkers and it catalyzes the conjugation of glutathione with xenobiotic, including perfluorinated compounds (Jemec et al.,2010). Oxidative stress and antioxidant enzymes are widely used as biomarkers in mussels. Mussels are sensitive to the effect of reactive oxygen species like other aerobic organisms (Winston et al., 1996; Funes et al., 2006). It has been shown that PFC type pollutants alter the levels of antioxidants in mussels (Liu et al., 2014). Glutathione S-transferase (GST) is the important phase II enzyme present in the living organisms. Glutathione S-transferase enzymes are dimeric proteins that have a key role in the detoxification of endogenous and exogenous electrophilic compounds (Mainwaring et al., 1996). Glutathione S-transferase activity measurements have been used in many different studies to monitor contamination level in different marine species. Many pollutants such as dichlorodiphenyl trichloroethane (DDT), Benzo[a]pyrene (BaP), perfluorooctanoic acid (PFOA), perfluorooctane sulfonate (PFOS). are detoxified by glutathione S-transferase enzymes.

The effects of persistent organic pollutants such as PFOS can occur at any different trophic levels. In aquatic toxicological studies to define the effects of toxicants, biomarkers are accepted as highly important tools. They have been used as early warning systems in order to protect environmental damages

In this study, the effects of Perfluorinated compounds (PFOS) on the induction of glutathione S-transferase activity in mussels were examined.

MATERIALS AND METHOD

Mussels were collected from the mussel culture facilities in İzmir-Çeşme, and acclimated to artificial seawater for 6 days. After the acclimation process, to evaluate the effect of GST enzyme activity, *Mytilus galloprovincialis* were exposed to different concentrations of PFOS. In order to test six concentrations of PFOS (2-3-5-6-8-10 ppm), a total of 120 mussels were used. During the experiment, *M. galloprovincialis* were fed daily by addition of 30 ml/l of *Chlorella* sp. (approximately 70,000 cells/mL) to each aquarium sized 57x39x28 cm. The mussels were kept by 12/12 light cycle. The water in the aquarium was changed every other day.

At the end of the experiment, the mussels were taken out and shell length and weight were measured by using calipper and digital scale [6,61 ± 0,41 (cm), 24,43 ± 5,01 (gr)]. To analyze the GST activity, hepatopancreas was dissected from the mussel as fast as possible and scaled. Collected tissues were washed with the phosphate buffer and homogenized in an Ultra Turax tissue homogenizer in homogenization buffer. 300 ml of homogenization buffer contained 1 M KCl (45 mL), 100 mM DTT (3 mL), 100 mM EDTA (3 mL), 10 mM PMSF (3 mL), 100 mM phosphate buffer (200 mL) and pH 7.4. The homogenate tissue was centrifuged at 10 000×g at 4°C for 30 min to obtain the postmitochondrial fraction. During the analysis, great attention is required to maintain the entire cold chain

GST activity was measured according to the method of Habig et al. (1974), by following the conjugation of reduced glutathione (GSH) with 1-chloro-2,4-dinitrobenzene (CDNB) at 340 nm for 10 min at constant temperature using kinetic spectrophotometer (BioTek-SYNERGY|HTX). Protein concentration was measured according to the method of Bradford (1976). GST activities were expressed as nmoles/min/mg of S10 protein (mg P). The differences between samples were investigated one-way ANOVA and Multiple Range Test. The statistical analysis was performed using the Statgraphics software v.16.2 and statistical significance was defined at $p<0.05$ level.

RESULTS

Hepatopancreas (digestive glands) were taken to determine the effect of various concentrations of PFOS on antioxidant enzyme activities of mussel, *M. galloprovincialis*. The results are presented in Figure 1 for all treatment periods including 7, 15 and 21 days. Levels of GST in control group were ranged between 0.023and 0.035 µmol/min/mg.

GST activity in hepatopancreas of mussels exposed to lowest concentration (2 mg/L) increased gradually from 0,027 to 0,044 µmol/min/mg between the 7th day and 21th day of experiment. Furthermore, in the mussels exposing to 3 mg/l PFOS concentration had the highest GST activity (0,074 µmol/min/mg) which was nearly twice of control value at the end of the 21th day of experiment The group exposed to 5 mg/l PFOS concentration showed similar pattern with slightly decreased value in the 21 day. The GST activity in the mussels exposed to 8 mg/l PFOS was higher nearly two times compared with control value in 7th day and the value decreased slightly in the next few days. On the other hand, the highest dosage of PFOS (10mg/l) caused the lowest GST activity in the mussel when compared to other experimental groups.

GST activity increasing regularly in lower concentrations was statistically significant ($p<0.05$). The results indicated that in lower concentrations of PFOS, GST activity can be increased. It means that organisms have healthy reaction to metabolizing the contaminants. However, in higher concentrations (8,10 mg PFOS/l), GST values changed irregularly comparing to control group ($p<0,05$). *M. edulis* samples exposed to 8 mg PFOS/l showed sharp increase during the first week of the test, but the level lowered gradually afterwards. Unlike to this, the group exposed to 10 mg PFOS/l had the weekest GST activity. This may explaine the toxic effect of PFOS on hepatopancreas of mussel in high concentrations. It seems that they couldn't have enough function to produce the enzymatic activity for protection. This result suggests that the toxic effect of PFOS is increased by restraining the production of enzyme

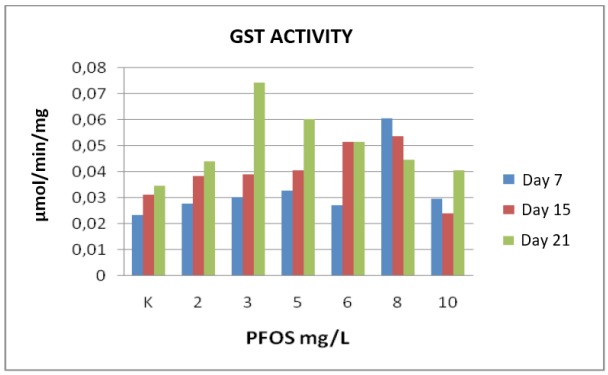

Figure 1. GST activity in the mussels affected by various concentrations of PFOS in the different test periods.

DISCUSSION

M. galloprovincialis has been commonly used as bioindicator organism in biomonitoring programs (Livingstone, 1998; Cheung et al., 2001; 2002). Because they are filter feeding organisms and they

bioaccumulate and biomagnify the pollutants (Zhou et al., 2008). Biomarker enzyme activities such as glutathione S-transferase and the other antioxidant enzymes have been studied in different mussel species (Cheung et al., 2004; Power and Sheehan, 1996; De Luca-Abbott et al.,2005).

Hepatopancreas of *M. galloprovincialis* were used in measurements of enzyme activity as biomarker. Because the hepatopancreas is the major metabolic organ for degrading xenobiotics and the first metabolic reactions on the molecules are achieved by the enzymes involved in biotransformation process in metabolism (Meyer,1996). Previous studies showed that GST in hepatopancreas tissue shows more utility for further investigation than gill tissue (De Luca-Abbott et al.,2005)

According to the results of this studies, GST activity (µmol/min/mg) in mussel exposed to low concentrations of PFOS tended to increase when it compared to the control group ($p < 0.05$). Similarly, positive correlations were observed between GST activities and POP's in the mussel, *M. galloprovincialis* collected from Bizerte Lagoon (Tunusia) where GST activity showed a trend of increase with increased toxicant concentration (Barhoumi et al.,2014). Fitzpatrick and Sheehan (1993) reported that GST enzyme activity can be useful indicator of mussel exposed to persistent organic pollutants.

Glutathione-Stransferases (GSTs) are the main enzymes involved in xenobiotic phase II metabolism. GSTs detoxify a number of environmental carcinogens (Gallagher et al., 1996). The measurement of glutathione S-transferase activity is also used as a biomarker of oxidative stress (Rodriguez-Ariza et al., 1993; Martínez-Gómez et al., 2006).

Organisms exposed to xenobiotics in various ways tend to convert chemicals into a more harmless form (Parlak et al., 2011). The induction of antioxidant enzyme activities represents a cellular defense mechanism to neutralize toxic effects of reactive oxygen species. There are many studies in which these activities are induced by pollutants presented in contaminated waters (Otto and Moon, 1995; Ferreira et al., 2005; Martínez- Gómez et al., 2006). In several studies, it has been shown that GST and some antioxidant enzyme activities have high sensitivity to contaminants in mussels (Power and Sheehan, 1996; Winston, et al.,1996). Gowland et al. (2002), reported that high moleculer weight PAHs have more pronounced role in inducing GST activity in mussels.

There are substantial amount of knowledges about the role of GST in aquatic species exposed to xenobiotics. The general aspect is that GST should have prominent position in MFO's as biomarker for ecotoxicological studies (Joselyn et al. 2012). In this study, the results of GST activities of mussel exposed to PFOS also propound that GST can be use as biomarker for studying the effect of pollutants especially in environmentally relevant concentrations.

CONCLUSION

In this study, mussel hepatopancreas exposed to PFOS in low concentrations, GST enzyme activity had showed a statistically significant increase when compared to the control group. The results showed that protective mechanisms were induced. On the other hand, in the mussels exposed to higher concentrations of contaminant this protective mechanism seemed to be depressed by the toxic action of PFOS. It is necessary to study the mode of toxic action of PFOS on the enzyme system in detail and determine long-term ecological effects of PFOS on aquatic organisms so as to gain important knowledge of the ecological risk of PFOS.

In addition, the susceptibility of *M. galloprovincialis* to various pollutants have been confirmed with toxicological studies. Moreover, the effects of various pollutants far below the lethal concentrations have been emphasized by determining changes in the physiological parameters of organism.

In conclusion, to protect the ecosystem, changes in enzyme activity due to the toxicants can be used as biomarkers. It is important to perform similar studies that will provide early warning signs for protection of species diversity and ecosystem health.

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